

Figure 1:

Amino acid sequences of Cpn60 and Cpn10:

SEQ ID No 1: Cpn10 (encoded by nucleotides pos. 458-751 of Figure 2):

MKIRPLHDRIVVRRKEEETATAGGHIIPGAAAEKPNQGVVISVGTGRILDNGSVQALA
VNEGDVVVFGKYSGQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 2: Cpn60 (encoded by nucleotides pos. 800-2446 of Figure 2):

MAAKDVLFGDSARAKMLVGVNIIADAVRVTLGPKGRNVVIEKSFGAPIITKDGVS
AREIELKDKFENMGAQMVKEVASQANDQAGDGTATVLAQAIISEGLKSVAAGMN
PMDLKRIGDKATAAVVAAIKEQAQPCLDTKAIAQVGTISANADEVGRILAEAMEKV
GKEGVITVEEGKGLEDEL DVVEGMQFDRGYLSPYFINNQEKM TVEMENPLILLVDKK
IDNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVVNNLRGTFKVA AVKAPGFGD
RRKAMLQDLAILTGQQVISEELGMSLETADPSSLGTASKVVIDKENTVIVDGAGTEAS
VNTRVDQIRAEIESSTSDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGVVAGGGVALIRALSSVTVVGDNEDQNVGIALALRAMEAPIRQI
AGNAGAEGSVVVDKVKSGTGSFGFNASTGEYGDMIAMGILDPAKVTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGGMGMPGMM

Figure 2:

SEQ ID No 3: DNA coding for Cpn60 and Cpn10:

Cpn10, pos. 458-751

Cpn60, pos. 800-2446

[illegible]

Figure 2 (continued):

cacaaccgaagccatgggtgcggatgcgcctgttgaagaaggcgcctgggtggtatgcctgatatgggcggcatgggtggaatgggcg
gtatgcctggcatgatgtaatcactttgtgattcattgtcctgatctgcctaccgtgtaaaaagatcaggctcaaggctgtctctataaaaag
ccgtatctttgatgagtgtgtcttctgtgtaaaacgacattcttggagtgcggccttttttgattttggtcataaaatcagaatatgtgtaatt
ttatgtaactagctggcctataatgttgagttcctctgggtggcatgatctcatggtacttcacttaagcctgattcactgag
gccttaacagtaaaataataacgcaacgtagaacaataaagcgtatggcattaatgaagacggctgcatttaattcagatc

Figure 3:

SEQ ID No 4: Amino acid sequence of esterase cloned from *Oleispira antarctica* (EstRB8):

EstRB8 (encoded by nucleotides 1145 to 2143 Frame 2 of Figure 4) 333 aa

MKNTLKSSSRFSLKQLGTGALHSSLFFGGCTTTQQDNLYTGVM SLARDSAGLEVKTA
SAGDVNLT YMERQGS DKDNAESVILLHGFSADKDNWILFTKEFDEKYHVIAVDLAG
HGDSEQLLT TDYGLIKQAERLDIFLSGLGVNSFHIAGNSMGG AISAIYSLSHPEKV KSL
TLIDAAGVDGDTESEYYKVLAEGKNPLIATDEASFEYRMGFTMTQPPFLPWPLRPSLL
RKTLARAEINN KIFSDMLKTKERLGMTNFQQKIEVKMAQHPLPTLIMWGKEDRVLD
VSAAA AFKKIIPQATVHIFPEVGH LPMVEIPSESAKVYEEFLSSIK

Figure 4:

SEQ ID No 5: DNA fragment from plasmid pBK1Est coding for esterase of *Oleispira antarctica* (EstRB8):

Nucleotide positions 1-100 correspond to reverse complement of positions 1196-1121 and 3799-3939 correspond to reverse complement of 1043-952 of pBK-CMV vector (Stratagene).

Positions 101-105 are *Bam*HI -- *Sau*3A1 fusion and positions 3795-3798 are *Sau*3A1-*Bam*HI-fusion.

acaggaaacagctatgaccttgattacgccaagctcgaaattaaccctcactaaaggaacaaaagctggagctcgcgcgcctgcag
gtcgacactagtggaalcaacggcggttcattggtactggctgagttcagcgctcataatgccgatgcgatactggccgtcagactgagtagt
tcttctgctagcaccgatttttctaafagcgcagcttcttatttctgaacgggcaactgattgtagtttttactaaccggcttttaggcaigg
taactcttcgatattcaaaattattactgttcataattacaatcatagtagcaggctagaggcccaaaattgcagctgatattcaccittattatc
taagcaattattacactcatcgcgggtgttatttaattgtgctaaataaaaatacccgtagcggaaaaattcagcaaatagccaaagaaaacga
ttggcaataaccaagaattcagcattttgatgatgacattaagcaggcaaaccttggcctattaaactacagtcaaaatgcaatttttagacat
ctcattcaagcaactgacgaacactatggcttagcgtttaagaccttggactgtcgcgcgttagaaccttcaggatttcacaatagcagctct
tattttatttaccctcgactaaagactgaattcaataacctacacatttgccttaagtcgacatattcaagataaagatgccctcactgacatca
gtcaccaacaatcaatcaaacaccaataccaatcgcaaaaaactcataaaactagccgatcaccaaatcccaaaaggcttcaaaaatgaa
acgagcacgctcacacaaaatcaattatacgttaacgaaccagggtcaaaactatcggtttttttagcagcgtttgttccactaatgaaagaga
aaagtcgttaattcactggcttttggcgatccgcaccttcacatagaaatagtaattggcatgctactggccttataaaagaatcagttaatt
gaagaaacctcgtttatctcagccattaccgctgtagccgaatttgcgcttacctcagccatgattaaactgcgcgaattaatataagac
atactaattaataacaccttaattgagaagaataatgaaaaacacactcaaatctcatcagcttttagctgaaacaactcggcaccggc
gctctgattatctccagtttcttctcgggtgggtgcaccacaacacaacaagataattatcacaggggttatgtctcttgcgagagacagc
gctggcctagaagfataaacagccctcgcgggtgacgtcaatcttactatfatggaacgccaaggcagtgacaaagataatgccgaag
cggtattttatcacgggttctctgctgataaagataactggattcttttaccaaagaattcgatgaaaaatacatgttalcgctgtcgattta
gggggacatggcgattcagaacaattatlaacgactgattacgggtctcataaaacaagccgagcgttttagatatcttctatctggcttagg
gggttaactcatttcacatcgccggttaattcaatggggggggctatcagcgcgaatctacagtttgagtcacccagagaaagttaaaagtctt
acattgatcgatgcagcaggtgtcgatggcgatactgaaagcgaatactacaaagttttggcagaaggtaagaatcctttaattgcaact
gatgaagcaagtttgaataccgcaltgggttcacatgactcagcctcttctaccttggccactaagaccttcttattacgtaaaacg
ctagcccggtccgagatcaataacaaaatttttccgatatgctgaaaaccaaagaacgtttaggaatgactaaccttcaacagaaaattg
aagtgaaaatggctcaacatccattgccaacactgattatgtggggcaagaagatcgcttcttgacgtatccgcagcagcgcccttc
aaaaaataattccacaagcaactgttcataatttctgaagtaggccacctacctaaggtagaaattcctagtgaagcgctaaagtttat

Figure 4 (continued):

gaagagttttgtctctattaaataagagcacataatcatgactgacttataaacagccaagcafttaaaatgcttggctgtttatttfaatgg
ccaaattattcaacgaccaagctcgcggtaaaatcgagtggtttctgttttcatcaacagcaacaaacgtgaaatacccgtaatcg
cattttctgattatcaaaatacactttccaccagcatatttaacttcaacttttaaaactgctccgccctacctctataacactggcagtcgaft
cgacaatggfacctgcgggaacaggatgcttaaaatcgattcgatcactgctgaecggttacgatgctttgtcgagaaaaacgagtcgct
gcaataaaagaaaccatccatccactgcatlgcagtgccacccaataacgtatcatgatgatttgtgtctcggaaataccgctttaga
aafagtgggttttgatacgcgctttcgtgcgcaataatattctctgctaagagttgcggatggcatacataaaactcgttgattaagatta
ataataaatagtaaacagtataatgaactgagggctgaagaacttcaaacctcgaagaacttgaggccgctagagagaaaagacca
gtgataaattttcatcttgccatgagagcttatcatgaagccctgtgcttaaaatcaatcaattatatttcatctttaaataaataaccaat
atatttcatataaatttccactacccttatctcactagacttcccgcgcatagggcgaacaataacgcaagttcacaataaagcgggttc
gctgcaacacatgccctagcgtctaaagtagcacgcacaacactggccagtcgactagcccttgcgattcgtgcagacgagcaac
aagegctattaaacttacctaaatttctaaccacacacatgggttctttccaaaaactcaaaaaaclegtcaaatccgcttgcaattttaaag
cgatgacatagatctaatcgattatcaaacccgcatcaagcgtcatltaaaaacgcaccactggcaagaagttctacctgcactgacca
atatgcaagcggcgccggaagagctgctttgatcgatcaagaagaaggagcagcaagaggaaaacaatcaaaaaggaggaga
gcaatcaataaaaaacgagttattgaggatttattttaaataaacagggtatataataacctctctcgtagtaaacatgactgtatttacaaa
aaataaataagaggatataccatgtcaaacatctggttgaagtaccaaagattgaagtattaaaccgtcaaatggaaaaactgcctgcagc
aaacttaggcattcaaaattacagaaattggcgatgatttatcactggcacaatgccagcagatgcacgtaccttcagccaatgggactg
attcatggcggtcaaatgtattgctggcagaaacactgggcagcatggcagctaactgctgtattatttgcctcaagaatattgtgtgg
ccaagaaattaacgccaaccacatacgcggtgttcgtccggcatagtactggcacagcaacgctagtagacaaaaggaagaacctc
ccagatttgggaaattcgcatcgtaacgatccaagaattcaaaaagcttctcgagagtacttctagagcggccgcccgcggccatcgati
ttccaccgggtgggggtaccaggtaaggttaaccaaitcgcctatagtgagtcgtattacaattcactggccgctgttttac

Figure 5:

Amino acid sequences expressed from vector pBK1CpnEst: - the co-expression of fragments encoding native chaperonins with the esterase gene (EstRB8), all from *Oleispira antarctica*

SEQ ID No 6: cpn10 (nucleotides 113 to 403; Frame 2 of Figure 6) 97 aa:

MKIRPLHDRIVRRKEETATAGGHLPGAAAEKPNQGVVISVGTGRILDNGSVQALA
VNEGDVVVFGKYSGQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 7: cpn60 (nucleotides 455 to 2098; Frame 2 of Figure 6) 548 aa:

MAAKDVLFGDSARAKMLVGVNLAADVRVTLGPKGRNVVIEKSFGAPIITKDGVS
AREIELKDKFENMGAQMVKEVASQANDQAGDGTATVLAQAIISEGLKSVAAGMN
PMDLKRIGDKATAAVVAAIKEQAQPCLDTKAIAQVGTISANADEVGRILAEAMEKV
GKEGVITVEEGKGLEDELVDVVEGMQFDRGYLSPYFINNQEKMTEMENPLILLVDKK
IDNLQELLPILENVAKSGRPLIVAEDVEGQALATLVVNNLRGTFKVAAVKAPGFGD
RRKAMLQDLAILTGGQVISEELGMSLETADPSSLGTASKVVIDKENTVIVDGAGTEAS
VNTRVDQIRAEIESSTSDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGVVAGGGVALIRALSSVTVVGDNEDQNVGIALALRAMEAPIRQI
AGNAGAEGSVVVDKVKSGTGSFGFNASTGEYGDMIAMGILDPKAVTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGGMGMPGMM

SEQ ID No 8: estRB8 (nucleotides 2579 to 3577; Frame 2 of Figure 6) 333 aa:

MKNTLKSSSRFSLKQLGTGALHSSLFEGGCTTTQQDNLYTGVMSLARDSAGLEVKTA
SAGDVNLTIMERQGSDDNAESVILLHGFSADKDNWILFTKEFDEKYHVIAVDLAG
HGDSEQLLTDDYGLIKQAERLDIFLSGLGVNSFHAGNSMGGASIAIYSLSHPEKVKSL
TLIDAAGVDGDTESEYYKVLAEKGKNPLIATDEASFEYRMGFTMTQPPFLPWPLRPSLL
RKTLARAEINNKIFSDMLKTKERLGMTNFQQKIEVKMAQHPLPTLIMWGKEDRVLD
VSAAAFAFKIIPQATVHIFPEVGHLPMEIPSESAKVYEEFLSSIK

Figure 6:

SEQ ID No 9: pBK1CpnEst: - the fusion of native chaperonine-coding fragments with
esterase of *Oleispira antarctica* (EstRB8)

The DNA fragment coding for Cpn10 and Cpn60 is flanked by *SacI* site (pos. 69-75) and *SalI*
site (encoded by pos. 2138-2143 of Figure 7):

Nucleotide positions 1-75 correspond to reverse complement of positions 1196-1121 and
positions 5233-5273 correspond to reverse complement of 1043-952 of pBK-CMV vector
(Stratagene)

Small letters – the Cpn10-Cpn60 encoding fragment,

Capital italics – fragments of vector pBK-CMV

Capital letters – fragment coding for EstRB8 from plasmid pBK1Est

ACAGGAAACAGCTATGACCTTGATTACGCCAAGCTCGAAATTAACCCCTCACTAAAGGGA
ACAAAAGCTGGAGCTCtaataacttgggatccaacagttggagagictagcaaatgaaaatccgtccattacatgatcgtatt
gttttcgccgtaaagaagaagagaccgcaactgcgggttggtattttaccgggcgtgcggcagaaaaaccaatcaaggtgtgt
tatctcgtgggtacttggcgtattcttataatgggtcagtgcaagcgtggcggttaacgaaggcgtgtgtcttttgtaaatactc
aggtcaaaatactatcgatcgtatgggaagaattatgtatttgaatgaaagtatactacggcgttttagaagcttaattattacactca
ctttttatitaacctacaaaatttaaggaaagatcagctgctaaagacgtattatttgggtatagcgcacgcgcacaaaatgttggtaggt
gtaaacatttttagcgcagcagtaagagttaccttaggacctaaaggctgtaacggtgttatagaaaaatcatttgggtgcaccgatcatcac
caaagatggtgtttctgttgcgcgtgaaatcgaattgaaagacaaatcgaaaacatgggcgcacagatggttaagggaagtgtcttca
agccaacgaccaagccgggtgacggcacaacgacagcgaactgtactagcacaggcgattatcagcgaaggcttgaatctgttgcgg
ctggcatgaatccaatggatctaaacgtgggtatlgataaagctacggcgtctgtgttgcggccattaaagaacaagctcagccttgcctg
gatacaaaagcaatcgtcaggtagggaacaactctgccaatgccgatgaacgggtggcgtttaattgctgaagcgaatggaaaaagt
cggtaagaagggtgtgattaccgtgaagaaggcaaaaggcctgaagacgagcttgatgtgtagaaggcatgcagttcgaatcgcgggt
actgtctccgtacttcatcaacaaccaagaaaaaatgaccgtagaatggaaaatccattaattctattggtgataagaaaattgataac
cttcaagagctgttgcgaattcttgaaaacgtcgttaaatcaggtcgtccattattgatcgttgcgaagatgttgaaggccaagcactagc
aacattggttagtaaacacttgcgcggcacattcaaggttcagcgggttaaaagccctgggtttggcgatcgtcgtaaagegatgtgca
agatcttgcacatctgacgggtggcaggttatttctgaagagctagggatgtctttagaaactgcggatccttcttcttgggtacggcaa
gcaaggtgttatcgataaagaaaacaccgtgatgttgatggcgaggtactgaagcaagcgttaatactcgtgttgaccagatccgtg
ctgaaatcgaaagctcgacttctgattacgacatcgaaaagttacaagaacgcgttgcctaaagcttgcggcgccggttgcctgattaag

Figure 6 (continued):

[illegible]

Figure 6 (continued):

CCTATGGTAGAAATTCCTAGTGAAAGCGCTAAAGTTTATGAAGAGTTTTTGTCTCT
CTATTAAATAAGAGCACATAATCATGACTGACTTATAAACAGCCAAGCATTAAAA
ATGCTTGGCTGTTTATTTTAATGGCCAAATTATTCAACGACCAAGCTCTGCGGTAA
AATCGCAGTGGGTTTCTTGTTCATCAACAGCAACAAACGTGAAATACCCCGTA
ATCGCATTTTTCTGATTATCAAAATACATACTTTCCACCAGCATATTAACCTCAAC
TTTTAAACTCGTCCGCCCTACCTCTATAACACTGGCAGTCAATTTCGACAATGGTAC
CTGCGGGAACAGGATGCTTAAAATCGATTTCGATCACTGCTGACGGTTACGATGCT
TTGTCGAGAAAAACGAGTCGCTGCAATAAAAGAAACCTCATCCATCCACTGCATT
GCAGTGCCACCGAATAACGTATCATGATGATTGTTGTCTCTGGAAATACCGCTTT
AGAAATAGTGGTTTTTGATACGCGCTTTCGCTGCGCAATAATATCTTCTCTGCTAA
GAGTTGCGGATGGCATACTAACTCGCTTGATTAAGATTAATAATAAATAGTTA
ACAGTATATTGAACTGAGGGTCTGAAGAACTCTAATACCTCTGAAGAACTTTGAG
GCCGCTAGAGAGAAAAGACCAGTGATAATATTTTCATCTTGCCATGAGAGCTTATC
ATGAAAGCCTGTGCTTAAAATCAATCATTATATTTATTCATCTTTAATTGAAATAA
TACCAATATATTTTCATATATAATTTTACACTACCTTATCTCACTAGACTTCCCGC
GCATAGGCGCAAACAATCAACGCAAGTTCACAATAAAGCGGTTTCGCTGCAACAC
ATGCCCTAGCGTCTAAAGTAGCACGCACAACACTGGCCAGTCGTACTAGCCCCTT
TGCGATTTCGTGCAGACGAGCAACAAGCGCTATTAAACTTACCTAAATTTCTAACC
ACCACCATTTGGTTCTTTTCCACAACTCAAAAACTCGTCAAATCCGCTTGCAATT
TAAACGCGATGACATAGATCTAATCGATTATCAAACCCGCATTCAAGCGCTCATT
AAAAACGCACCACTGGCAAGAAGTTCTACCTGCACTGACCAATATGCAAGCGGC
GGCGGAAGAGCTGCCTTTGATCGATCAAGAAGAAGGGAGCAGCAAAGAGGAAA
ACAATCAAAAAGAGGAGAGCAATCAAATAAAAAACGAGTTATTGAGGATTTTAAT
TTTAAACAGGTATATTAATACCCTCTCTCGTAGFAACAATGACTGTATTTACAC
AAAAATAAATAGAGGTATACCATGTCAAACATCTGGTTTGAAGTACCAAAGATTG
AAGTATTAAACCGTCAAATGGAAAATACTGCCTGCAGCAACTTAGGCATTCAAAT
TACAGAAATTGGCGATGATTATCACTGGCACAATGCCAGCAGATGCACGTACC
TTCCAGCCAATGGGACTGATTCATGGCGGCTCAAATGTATTGCTGGCAGAAACAC
TGGGCAGCATGGCAGCTAACTGCTGTATTAATTTGTCTCAAGAATATTGTGTTGG
CCAAGAAATTAACGCCAACCACATACGCGGTGTTTCGTTCCGGCATAGTGACTGGC
ACAGCAACGCTAGTACACAAAGGAAGAACCTCCCAGATTTGGGAAATTCGCATC

Figure 6 (continued):

GTTAACGATCCAAAGAATTCAAAAAGCTTCTCGAGAGTACTTCTAGAGCGGCCGCGGG
CCCATCGATTTTCCACCCGGGTGGGGTACCAGGTAAGTGTACCCAATTCGCCCTATAGT
GAGTCGTATTACAATTCACCTGGCCGTCGTTTTAC

Figure 7:

Amino acid sequences expressed from vector pBK1CpnSREst: - the co-expression of the stabilized single ring mutant chaperonin with the esterase gene (EstRB8) from *Oleispira antarctica* (cpn10::stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala::est)

SEQ ID No 10: cpn10 (nucleotides 113 to 403; Frame 2 of Figure 8) 97 aa:

MKIRPLHDRVVRRKEEETATAGGIILPGAAAEKPNQGVVISVGTGRILDNGSVQALA
VNEG DVVVF GKYS GQNTIDIDGEELLILNESDIYGVLEA

Below – ***Capital bold letters*** are the mutations introduced

SEQ ID No 11: stabilized single ring mutant of cpn60 (nucleotides 455 to 2098; Frame 2 of Figure 8) 548 aa:

MAAKDVLFGDSARAKMLVGVN~~IL~~ADAVRVT~~L~~GPKGRNVVIEKSFGAPIITKDGVSV
AREIELKDKFENMGAQMVKEVASQANDQAGDGT~~TT~~TATVLAQAIISEGLKSVAAGMN
PMDLKR~~G~~IDKATAAVVAAI~~KE~~QAQPC~~L~~DTKAIAQVGTISANADET~~V~~GR~~L~~IAEAMEKV
GKEGVITVEEGKGLEDEL~~D~~VVEGMQFDRGYLSPYFINNQE~~K~~MTVEMENPLILLVDKK
IDNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVVNNLRGTFKVA~~AV~~KAPGFGD
RRKAMLQDLAILTGGQVISEELGMSLETADPSSLG~~T~~ASKVVIDKENTVIVDGAGTEAS
VNTRVDQIRAEIESSTSDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGVVAGGGVALIRALSSVT~~V~~VGD~~N~~EDQNVGIALALRAMEAPIRQI
AGNAGA~~AG~~~~AA~~VVDKVKSGTGSFGFNASTGEYGD~~M~~IAMGILDP~~AK~~VTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGGM~~PG~~MM

SEQ ID No 12: EstRB8 (nucleotides 2579 to 3577; Frame 2 of Figure 8) 333 aa:

MKNTLKSSSRFSLKQLGTGALIISS~~L~~FFGGCTTTQQDNLYTGVM~~S~~LARDSAGLEVKTA
SAGDVN~~L~~TYMERQGS~~D~~KDNAESVILLHGFSADKDNWILFTKEFDEKYHVIAVDLAG
HGDSEQLLT~~T~~DYGLIKQAERLDIFLSGLGVNSFHIAGNSMGG~~A~~ISAIYSLSHPEKVKSL

Figure7 (continued):

TLIDAAGVDGDTSEYYKVLAEKGKNPLIATDEASFEYRMGFTMTQPPFLPWPLRPSLL
RKT LARAEINN KIFSDMLKTKERLGMTNFQQKIEVKMAQHPLPTLIMWGKEDRVLD
VSAAAFAFKKIIPQATVHIFPEVGH LPMVEIPSESAKVYEEFLSSIK

Figure 8:

SEQ ID No 13: DNA sequence of vector pBK1CpnSREst: the expression cassette for the co-expression of the stabilized single ring mutant chaperonin with the esterase gene (EstRB8) from *Oleispira antarctica* (cpn10::stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala::est)

Nucleotide positions 1-75 correspond to reverse complement of positions 1196-1121 and positions 5233-5273 correspond to reverse complement of 1043-952 of pBK-CMV vector (Stratagene)

DNA fragment coding for Cpn10 and Cpn60 is flanked by *SacI* site (pos. 69-75) and *Sall* site (pos. 2138-2143).

In the DNA sequence:

Small letters --- the Cpn10-Cpn60 coding fragment,

Capital italics --- fragments of vector

Capital letters -- fragment coding for EstRB8 from plasmid pBK1Est

Capital bold letters = introduced mutations

ACAGGAAACAGCTATGACCTTGATTACGCCAAGCTCGAAATTAACCTCACTAAAGGGA
ACAAAAGCTGGAGCTCtaataacttgggaaccaacagttggagagcttagcaaatgaaaatcgcgcattacatgaicgtt
gttggtcgcgtaagaagaagagaccgcaactgcgggtggtattatfttaccggcgctgcggcagaaaaaccaaataagggtgtgt
taletctgtgggtactggccgtatcttgataatgggtcagtgcaagcgcgtggcggttaacgaaggcgatgttgtcgttttggtaatactc
aggtaaaaatactatcgatcgttggtgaagaattatgtatgaatgaaagtgaatctacggcggtttagaagcctaattattacactca
ctttttatttaacctacaaaatttaaggaaagatcatggctgctaaagacgtattatitgggtgatagcgcacgcgcaaaaatgttggtaggf
gtaaacattttagccgacgcagtaagagttaccitaggacctaaggctgtaacgttggtatagaaaaatcatttggtgcaccgafcatcac
caaagatgggtgtttctgttgcgctgaaatcgaaatgaaagacaaatcgaaaacatgggcgcacagatggtaagggaagttgcttctca
agccaacgaccaagccggtgacggcacaacgacagcgactgtactagcacaggcgattatcagcgaaggcttgaatctgttgcgg
ctggcatgaatccaatggatcttaaacgttggtatgataaagctacggctgctgtgttgcggccattaaagaacaagctcagccitgcttg
gatacaaaaagcaatcgctcaggtagggaacaatctctgccaatgccgatgaacgggttggtcgtttaattgctgaagcgatggaaaaagt
cggtaaagaaggtgtgattaccgttgaagaaggcaaggccttgaagacgagcttgatgtttagaaggcatgcagttcgtcgcgggt
actgtctccgtacttcatcaacaaccaagaaaaaatgaccgtagaaatggaaaaatcatiaattctattggttgataagaaaattgataac
cttcaagagctgttgccaattctgaaaacgttcgctaaatcaggtcgtccattatgtatcgttgcgaagatgttgaaggccaagcactagc

Figure 8 (continued):

aacattggtagtaaacacttgcgcggcacattcaaggtgcagcggtaaaagcccctggtttggcgatcgctglaaagcgatgttgc
agatcttgcacatctgacgggtggcagggtatttctgaagagctagggatgtcttagaaactgcggatccttcttcttgggtacggcaa
gcaagggtgtatcgataaagaaaacaccgtgattgtgatggcgagggtactgaagcaagcgttaatactcgtgttgaccagatccgtg
ctgaaatcgaaagctcgacttctgattacgacatcgaaaagtacaagaacgcgttgctaaagcttgcggcgccggttgccgtgattaag
gttgggtgcgggttctgaaatggaatgaagagagaagaaagaccgtgtgacgatgcacttcacgaactcgcgcagcgggtgaagaag
gtgtgttgcgggtggtggtgtgtcttggatcgcgcactctctcagtaaccgtgtgtgtgataacgaagatcaaaacgtcgggtattgcat
tggcacttcgtgcgatggaagctcctalcgicaaaatcgcggttaacgcagggtgctgCagggGcagCggttgtgataaagtgaat
ctggcacaggtagcttgggttaacgccagcacagggtgagtatggcgatgatgttgcgatgggtatttagacctgcaaaagtcacgc
gttcactctacaagccgcggcgctatcgagggttgatgatcacaccgaagccatggttgcggatgcgcctgttgaagaaggcgct
gggtgatgctgatggggcgcatgggtggaatggcggtatgcctggcatgatgtaacacttgtgtatcattgtctctgactgtta
ccgtGTTCGACATATTCAAGATAAAGATGCCTTCACTGACATCAGTCACCAACAATC
AATCAAACACCAATACCAATCGCAAAAACCTCATAAACTAGCCGATCACCAAAT
CCCAAAAGCGTTCAAAAATGAAACGAGCACGTCACACAAAATCAATTTATACGC
TAACGAACCAGGTCAAACCTTATCGTTTTTTTGAGCACGTTTGTTCCTACTAATGAAA
GAGAAAAGTCGTTAATTCAGTGGCTTTTGGCGTATCCGCACCTTCACATAGAAAT
TAGTAATGGCATGCTACTGGCCTTTAAAAAGAATCAGTTAATTGAAGAAACCTCG
CTTATCTCAGCCATTACCGCTGTAGCCGAATTTGCGCTTATCCTCAGCCATGATTA
AACTGACGCCAATTAATATAAGACATACTAATTAATAACTCCCTTAATTGAGAAG
AATAATGAAAAACACACTCAAATCCTCATCACGTTTTAGTCTGAAACAACCTCGGC
ACCGGCGCTCTGATTATCTCCAGTTTGTCTTTCGGTGGTTGCACCACAACACAACA
AGATAATTTATACACAGGGGTTATGTCTCTTGCAGAGAGACAGCGCTGGCCTAGAA
GTTAAACAGCCTCTGCCGGTGACGTCAATCTTACTTATATGGAACGCCAAGGCA
GTGACAAAGATAATGCCGAAAGCGTTATTTTATTACACGGTTTCTCTGCTGATAA
AGATAACTGGATTCTTTTTACCAAAGAATTCGATGAAAAATATCATGTTATCGCT
GTCGATTTAGCGGGACATGGCGATTCAGAACAAATFATTAACGACTGATTACGGTC
TCATAAAACAAGCCGAGCGTTTAGATATCTTCTTATCTGGCTTAGGGGTAACTC
ATTTACATCGCCGGTAATTCAATGGGGGGGGCTATCAGCGCAATCTACAGTTTG
AGTCAACCAGAGAAAGTTAAAAGTCTTACATTGATCGATGCAGCAGGTGTCGATG
GCGATACTGAAAGCGAATACTACAAAGTTTTGGCAGAAGGTAAGAATCCTTTAAT
TGCAACTGATGAAGCAAGTTTTGAATACCGCATGGGTTTCACCATGACTCAGCCT
CCTTTCTACCTTGGCCACTAAGACCTTCTTTATTACGTAAAACGCTAGCCCGTGC
CGAGATCAATAACAAAATTTTTTCCGATATGCTGAAAACCAAAGAAGCTTTAGGA

Figure 8 (continued):

ATGACTAACTTTCAACAGAAAATTGAAGTGAAAATGGCTCAACATCCATTGCCAA
CACTGATTATGTGGGGCAAAGAAGATCGCGTTCTTGACGTATCCGCAGCAGCGGC
CTTCAAAAAAATAATTCCACAAGCAACTGTTTCATATTTTTCTGAAGTAGGCCAC
CTACCTATGGTAGAAATTCCTAGTGAAAGCGCTAAAGTTTATGAAGAGTTTTTGT
CCTCTATTAAATAAGAGCACATAATCATGACTGACTTATAAACAGCCAAGCATTT
AAAATGCTTGGCTGTTTATTTTAATGGCCAAATTATTCAACGACCAAGCTCTGCG
GTAAAATCGCAGTGGGTTTCTTGTTTTTCATCAACAGCAACAAACGTGAAATACCC
CGTAATCGCATTTTTCTGATTATCAAAATACATACTTTCCACCAGCATATTAACTT
CAACTTTTAAACTCGTCCGCCCTACCTCTATAACACTGGCAGTCAATTCGACAATG
GTACCTGCGGGAACAGGATGCTTAAAATCGATTTCGATCACTGCTGACGGTTACGA
TGCTTTGTGAGAAAAACGAGTCGCTGCAATAAAAGAAACCTCATCCATCCACTG
CATTGCAGTGCCACCGAATAACGTATCATGATGATTTGTTGTCTCTGGAAATACC
GCTTTAGAAATAGTGGTTTTTGATACGCGCTTTCGCTGCGCAATAATATCTTCTCT
GCTAAGAGTTGCGGATGGCATAACATAAACTCGCTTGATTAAGATTAATAATAAAT
AGTTAACAGTATATTGAACTGAGGGTCTGAAGAACTCTAATACCTCTGAAGAACT
TTGAGGCCGCTAGAGAGAAAAGACCAGTGATAATATTTTCATCTTGCCATGAGAGC
TTATCATGAAAGCCTGTGCTTAAAATCAATCATTATATTTATTCATCTTTAATTGA
AATAATACCAATATATTTTCATATATAATTTTCACACTACCCTTATCTCACTAGACTT
CCCGCGCATAGGCGCAAACAATCAACGCAAGTTCACAATAAAGCGGTTTCGCTGC
AACACATGCCCTAGCGTCTAAAGTAGCACGCACAACACTGGCCAGTCGTACTAGC
CCCTTTGCGATTTCGTGCAGACGAGCAACAAGCGCTATTAAACTTACCTAAATTC
TAACCACCACCATTGGTTCTTTTCCACAACTCAAAAAACTCGTCAAATCCGCTTG
CAATTTAAACGCGATGACATAGATCTAATCGATTATCAAACCCGCATTCAAGCGC
TCATTA AAAACGCACCACTGGCAAGAAGTTCTACCTGCACTGACCAATATGCAAG
CGGCGGCGGAAGAGCTGCCTTTGATCGATCAAGAAGAAGGGAGCAGCAAAGAGG
AAAACAATCAAAAAGAGGAGAGCAATCAAATAAAAACGAGTTATTGAGGATTTT
AATTTTAAAACAGGTATATTAATACCCTCTCTCGTAGTAAACAATGACTGTATTTA
CACAAAAATAAATAGAGGTATACCATGTCAAACATCTGGTTTGAAGTACCAAAG
ATTGAAGTATTAAACCGTCAAATGGAAAATACTGCCTGCAGCAACTTAGGCATTC
AAATTACAGAAATTGGCGATGATTATATCACTGGCACAATGCCAGCAGATGCACG
TACCTTCCAGCCAATGGGACTGATTCATGGCGGCTCAAATGTATTGCTGGCAGAA
ACACTGGGCAGCATGGCAGCTAAC TGCTGTATTAAATTTGTCTCAAGAATATTGTG

Figure 8 (continued):

TTGGCCAAGAAATTAACGCCAACCACATACGCGGTGTTTCGTTCCGGCATAGTGAC
TGGCACAGCAACGCTAGTACACAAAGGAAGAACCTCCCAGATTTGGGAAATTCG
CATCGTTAACGATCCAAAGAATTCAAAAAGCTTCTCGAGAGTACTTCTAGAGCGGCCG
CGGGCCCATCGATTTTCCACCCGGGTGGGGTACCAGGTAAGTGTACCCAATTCGCCCT
ATAGTGAGTCGTATTACAATTCACCTGGCCGTCGTTTTAC

Figure 9:

Amino acid sequence of the stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala of Cpn60:

SEQ ID No 14: Cpn10 (nucleotides 458-751 of Figure 10):

MKIRPLHDRVVRREEETATAGGHLPGAAAEKPNQGVVISVGTGRILDNGSVQALA
VNEGDVVVFGKYSGQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 15: Cpn60 (nucleotides 458-751 of Figure 10):

MAAKDVLFGDSARAKMLVGVNLAADAVRVTLGPKGRNVVIEKSFGAPIITKDGVSV
AREIELKDKFENMGAQMVKEVASQANDQAGDGTTTATVLAQAIHSEGLKSVAAGMN
PMDLKRIGDKATAAVVAAIKEQAQPCLDTKAIAQVGTISANADEVGRILAEAMEKV
GKEGVITVEEGKGLEDEL DVVEGMQFDRGYLSPYFINNQEKM TVEMENPLILLVDKK
IDNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVVNNLRGTFKVA AVKAPGFGD
RRKAMLQDLAILTGGQVISEELGMSLETADPSSLGTASKVVIDKENTVIVDGAGTEAS
VNTRVDQIRAEIESSTSDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGVVAGGGVALIRALSSVTVVGDNEDQNVGIALALRAMEAPIRQI
AGNAGAAGAAVVVDKVKSGTGSFGFNASTGEYGDMIAMGILDPKAVTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGGMGMPGMM

Figure 10:

SEQ ID No 16: DNA sequence of the stabilized single ring mutant
Glu460Ala/Ser462Ala/Val463Ala:

In the DNA sequence:

Small letters – the Cpn10-Cpn60 coding fragment,

Big bold letters = introduced mutations

atcaaaaaatgcagcaaggacagattcctgccccagaatagcagaagggttcttgttagcaclggccggcgcttaltatfaacgccgg
gtttgtcactgatgcgctgggtttacattactegtecccgacgcgtaaaagcggtggtccataagggtgattgcattattaccctc
gcatgatgactgcaagcagcttcaagcgacgggtagtttcaggaaggctcgttfaaagatgtacattcgacacagctcgaagca
gtcatgaaaaaatcacaattgaaggcgaatataccaaagacgataagtaggtatttttggctagccggtgaaatcctagtaaaagccc
cgataaaitaaccatctattttcacagaggcaatttagcccttgtttacattatgacttaatacttgggacccaacagttggagagctfagc
aaatgaaaatccgtccattacatgatcgtattgttgcgcgtaaaagaagaagagaccgcaactgcgggtggtattatttacc
gggcgtgcggcagaaaaacaaatcaagggtgtttatctctgtgggtactggccgtattcttgataatggttcagtgcgaagcgctggc
ggttaacgaaggcgatgttgcgttttggtaatactcaggtcaaaatactatcgatafcgatggtgaagaaitattgatttgaatga
aagtgatactacggcggtttagaagcttaattattacactcacctttttttaacctacaaaaittaaggaaagatcatggtgctaaagacg
tattatttgggtgatagcgacgcgcaaaaatgttggtgagggtgtaaacattttagccgacgcagtaagagttaccttaggacctaa
aggctgtaacgttgttatagaaaaatcatttgggtgcacccgacacaccaaagatgggtgttctgttgcgcgtgaaatcgaattgaaagaca
aatcgaaaacatgggcgcacagatggttaagggaagttgcttctcaagccaacgaccaagccgggtgacggcacaacgacagcgact
gtactagcacaggcgattatcagcgaaggcttgaatctgttgcggctggcatgaafccaatggatcttaaacgtgggtattgataaagcta
cggctgctgttgttgcgcccaataagaacaagctcagccttgcgttgatacaaaagcaatcgctcaggtaggggacaatctctgccaatg
ccgatgaaacgggttggcgtttaattgtgaagcgatggaaaagtcggtaaaagaggtgtgattaccgttgaagaaggcaaggcctt
gaagacgagcttgatgtttagaaggcaatgcagttcgatcgcggtacttctcctgacttcaacaaccaagaaaaatgaccgta
gaaatggaaaatccattaatctatgttgggtgataagaaaatgataacctcaagagctgttgcgaattcttgaaaacgfcgctaaatcaggt
cgfccattattgatcgttgtgaagatgtgaaggccaagcactagcaacattggtagtaacaacttgcgggcacattcaaggttgc
agcggttaaaagccccctgggttggcgatcgtcgtaaagcgatgttgaagatcttgcacattgacgggtggtcaggttattctgaagag
ctagggatgtcttagaaaactcgggactcttcttgggtacggcaagcaagggtgttatcgataaagaaaaacaccgtgattgttga
tggcgcagggtactgaagcaagcgtaataactcgtgttgaccagatccgtgtgaaatcgaaagctcgacttctgattacgacatcgaaaa
gttacaagaacgggttgcataagcttgcggggcggttgcgtgaltaaagggttgggtcgggttctgaaatggaaatgaaagagaagaaa
gaccgtgttgacgatgcacttcatgcaactcgcgcagcggtgaagaagggtgttgttgcgggtgggtgtgttcttatttcgcgcactct
cttcagtaaccgttgttgggtgataacgaagatcaaacgtcggtattgcatlggcacttctgtcgatggaagctcctatcgcgtaaaatgcg

Figure 10 (continued):

gggtaacgcaggtagctgCagggGcagCgggtgtgataaagtgaatctggcacaggtagctttggtttaacgccagcacaggtag
agtatggcgatatgatggcatgggtattttagacctgcaaaagtcacgcgttcactctacaagccggcggtctatcgccaggtttgat
gatcacaaccgaagccatgggtggcgatggcctgtgaagaaggcgctgggtggtatgcctgatatggcgggcatgggtggaatggg
cgggtatgctggcatgatgtaatcactttgtgattcattgtcctgatctgctaccgtgtaaaaagatcagggtcaaggctgtctctataaaa
agccglatctttgatgagtggtgtcttctgtctgaaaacgacattctggagtgccgcttttttgattttggtcataaaatcagaatattgtgta
atttatgtaactagctggcctataatgttgagttcctctgggtggcatgatctcatggtaacttcaactaagcctgattcaactgag
gctttaacagtaaaataataacgcaacgtagaaacataataagcgatggcattaatgaagacggctgcatttaattcagatc